5-HT_3 Receptor Antagonists. 3. Quinoline Derivatives Which May Be Effective in the Therapy of Irritable Bowel Syndrome

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A series of quinolinecarboxylic acid derivatives has been previously described as a new class of 5-HT_3 receptor antagonists due to deviation of a carbonyl moiety from the plane of an aromatic ring in their minimum-energy conformations. These derivatives were evaluated in a wrap-restraint stress-induced defecation model in rats. Reference compounds, ondansetron (**1**), granisetron (**2**), and YM060 (**4**), potently inhibited a stress-induced increase in stools excreted from fed rats (ID_{50} = 0.27, 0.12, and 0.0052 mg/kg, po, respectively). However, quinoline derivatives exhibited different activities depending on structural class. 4-Hydroxyquinoline-3-carboxylic acid derivatives **5** and **6a** possess high affinity for the 5-HT_3 receptor (K_i = 6.1 and 1.5 nM, respectively) and exhibit potent activity in the Bezold–Jarisch (B-J) reflex test (ED_{50} = 0.0017 and 0.000 10 mg/kg, iv, respectively), but they did not effectively inhibit the increase in fecal pellet output at the dose of 1 mg/kg, po. On the other hand, most of 1-substituted 2-oxoquinoline-4-carboxylates **10** showed less potent activity in the B-J reflex test than **1** or **2** but inhibited restraint stress-induced defecation more potently than **1** or **2**. The ID_{50} value of *endo*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 1-isobutyl-2-oxo-1,2-dihydro-4-quinolinecarboxylate **10e** was 0.013 mg/kg, po. With respect to the selected compounds **6a** and **10e**, effects on 5-HT- and thyrotropin-releasing hormone (TRH)-induced defecation, castor oil-induced diarrhea and wrap-restraint stress-induced colonic propulsion in rats were examined. These 5-HT_3 receptor antagonists did not effectively inhibit castor oil-induced diarrhea, which has been reported not to be mediated via the 5-HT_3 receptor. Although **10e** showed 800-fold decreased potency compared with **4** in the B-J reflex test, **10e** exhibited activity as potent as **4** in 5-HT- and TRH-induced defecation assays; **10e** exhibited 7-fold increased potency compared with **4** in wrap-restraint stress-induced colonic propulsions. From these results, **10e** appears to interact selectively with 5-HT_3 receptors in the gastrointestinal system and might be effective in the therapy of irritable bowel syndrome (IBS).

Introduction

5-Hydroxytryptamine (5-HT) is a biogenic amine that mediates a variety of physiological actions by binding to distinct cell-surface receptors.[1] 5-HT receptors have been classified into 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 subtypes on the basis of their pharmacological and physiological responses.[2-5] Recently, the 5-HT_3 receptor has attracted considerable attention, and our understanding of this receptor has increased dramatically over the past few years because of the discovery and widespread availability of potent and selective antagonsits.[1] These antagonists include ondansetron (**1**),[6] granisetron (**2**),[7] and tropisetron (**3**),[8] which have been shown to be effective in the control of cancer chemotherapy-induced emesis, an event suggested to be modulated by the 5-HT_3 receptor in the area postrema.[9]

Approximately 90% of endogenous 5-HT is estimated to be within the gastrointestinal tract, and the 5-HT_3 receptor was originally found in the periphery.[10,11] The role of 5-HT_3 receptors in controlling gastrointestinal contractility in vitro is well established. 5-HT or the selective 5-HT_3 receptor agonist, 2-methyl-5-HT, stimulates contractile responses of guinea pig ileum[12,13] or colon[14,15] which are blocked by 5-HT_3 receptor antagonists. Furthermore, the fact that immobilization stress results in an increase in plasma and brain 5-HT concentration in rats indicates the possibility that 5-HT acts as a mediator of gut dysfunction caused by stress. Recently, in vivo examination also showed the 5-HT acted as a mediator of restraint stress-, 5-HT-, or thyrotropin-releasing hormone (TRH)-induced gut dysfunction through the 5-HT_3 receptor.[10,11,16] A potent and selective 5-HT_3 receptor antagonist, YM060 (**4**)[15] was reported to inhibit restraint (cage) stress-, 5-HT-, and TRH-induced defecation and diarrhea.[16] A recent study by Williams et al. indicates that there are similarities between the intestinal effects of wrap-restraint stress in rats and intestinal symptoms associated with stress and irritable bowel syndrome (IBS) in humans.[17] Thus, wrap-restraint may be an appropriate animal model in which to study stress-related intestinal dysfunction. On the other hand, stress-induced transit of solid residue through the proximal colon has been demonstrated to be a determinant of stool weight in patients with diarrhea-predominant IBS.[18] In fact, ondansetron (**1**) is reported to slow colonic transit in healthy humans and is under clinical trial as a drug for diarrhea predominent IBS.[18b-d] Thus, it is of great interest for new drug development to determine whether 5-HT_3 receptor antagonists inhibit wrap-restraint stress-induced defecation and/or accelerated transit through the colon.

We have recently reported novel quinoline derivatives as 5-HT_3 receptor antagonists (**5–13**), of which binding affinities did not correlate with in vivo activities [Bezold–Jarisch (B-J) reflex test].[19,20] The selective 5-HT_3 receptor antagonists reported to date may be represented by a

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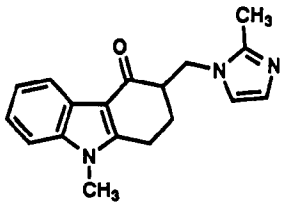

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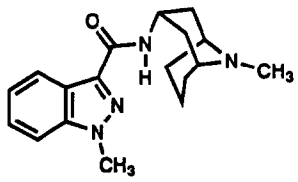

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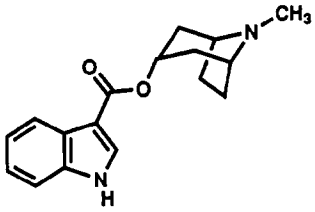

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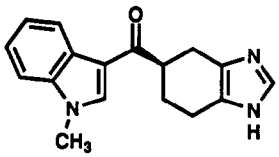

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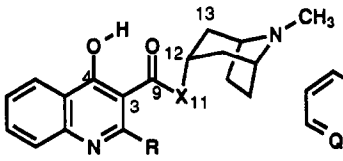
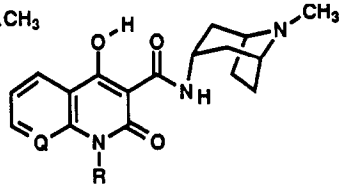


5 (X = O, R = H)
6 (X =NH)



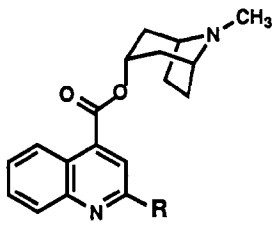
7 (Q = CH)
8 (Q = N)

combination of an aromatic group and a carbonyl-containing moiety connected to a basic amine. The carbonyl group is coplanar to an aromatic group, and interatomic distances in the 5-HT$_3$ receptor antagonist pharmacophore are in adequate ranges (O in a carbonyl group–an aromatic center, ca. 3–4 Å; O in a carbonyl group–N in a basic center, ca. 5 Å; N in a basic center–an aromatic center, ca. 7–8 Å).[21-24] Molecular modeling studies using QUANTA 3.2/CHARMm 21.3 revealed that although the interatomic distances in the pharmacophores of our quinoline derivatives were similar to the reported values, the carbonyl moiety in the minimum-energy conformers of **7c** (R – CH_3), **10a** (R = CH_3), and **11d** (R = CH_3) deviated from the plane of an aromatic ring.[19,20] The preceding 5-HT$_3$ receptor antagonists such as **14** and **15** possessed planar conformation.[19,20] Therefore, we speculated that our quinoline derivatives might interact with the 5-HT$_3$ receptor in a different way from that of the reported 5-HT$_3$ antagonists.

This paper describes the effects of these quinoline derivatives on wrap-restraint stress-induced defecation and transit through the colon and their possibility in the therapy of IBS.

Chemistry

Synthetic methods of most of the compounds have been previously described.[19,20] Compound **9a** was prepared as shown in Scheme I. Isatin (**16**) was reacted with 4-methyl-2-oxopentane to give the carboxylic acid **17a**, which was then treated with $SOCl_2$ followed by reaction with tropine to afford the target compound **9a**. Compound **9b** was similarly prepared from 2-phenylquinoline-4-carboxylic acid (**17b**) (Scheme II). The physical and analytical data of the newly synthesized compounds are listed in Table I. Ondansetron (**1**),[25] granisetron (**2**),[26] and YM060 (**4**)[27] were prepared by using the reported procedures, respectively.



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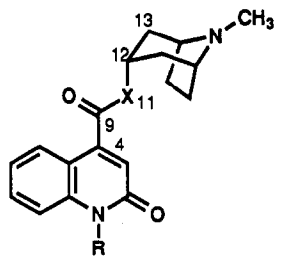
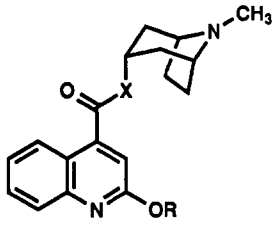
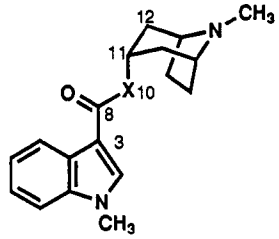


10 (X = O)
11 (X = NH)



12 (X = O)
13 (X = NH)



14 (X = O)
15 (X = NH)

Results

Wrap-Restraint Stress-Induced Defecation in Rats. Wrap-restraint stress (1 h) increased stool excretion 3–5-fold more than that in the normal rat. As described in introduction, 5-HT is reported to act as a mediator of restraint (cage) stress-induced gut dysfunction through the 5-HT$_3$ receptor.[16] Thus, our quinoline-type 5-HT$_3$ antagonists were evaluated in a wrap-restraint stress-induced defecation model. The results are shown in Tables II and III along with data of the binding assay and the B-J reflex test.[19,20]

Oral administration (1 h treatment) of **1**, **2**, and **4** dose-dependently inhibited wrap-restraint stress-induced increases in stools excreted from fed rats. The ID_{50} values of **1**, **2**, and **4** were 0.27, 0.12, and 0.0052 mg/kg, respectively (Table II). Although 4-hydroxyquinoline-3-carboxylic acid derivatives (**5–7**) possess high affinity for the 5-HT$_3$ receptor and exhibit potent activity in the B-J reflex test, they did not effectively inhibit the increases in fecal pellet output at the dose of 1 mg/kg. The ID_{50} value of **6a** (KF 20170) was 1.4 mg/kg, which was 270-fold larger than that of **4**.

Subsequently, we evaluated quinoline-4-carboxylic acid derivatives in this model. 2-Substituted 4-quinolinecarboxylates **9a** and **9b** also did not effectively inhibit fecal pellet output at the dose of 1 mg/kg (Table II). However most of 1-substituted 2-oxo-1,2-dihydroquinoline-4-carboxylates (**10b–10i**) inhibited wrap-restraint stress-induced defecation more potently than ondansetron or granisetron (Table III). Among them, compounds with a branched alkyl chain at the 1-position exhibited the most potent activity (**10c**, **10e**, and **10g**). On the other hand, the amide derivatives (**11a–11c**), with dramatically decreased binding affinity for the 5-HT$_3$ receptor and activity in the B-J reflex test, did not effectively inhibit wrap-restraint stress-induced defecation. Further, 2-alkoxyquinoline-4-carboxylates (**12a-12f**), which possess high affinity for the 5-HT$_3$ receptor and exhibit moderate activity in the B-J reflex test, did not effectively inhibit defecation. 2-Alkoxyquinoline-4-carboxamide derivatives (**13a–13c**), which possess low affinity for the 5-HT$_3$ receptor, did not effectively inhibit defecation at the dose of 1 mg/kg.

Scheme I

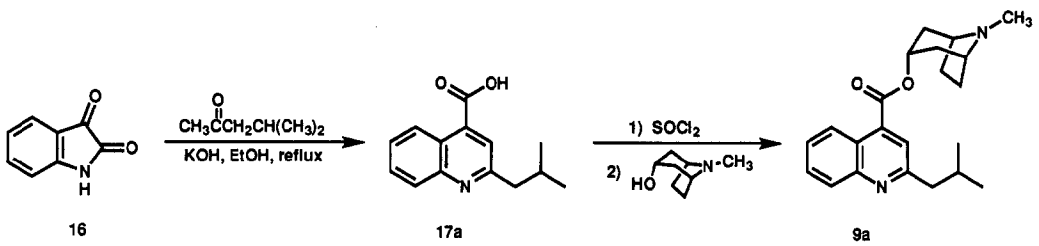
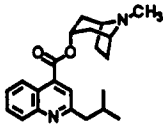
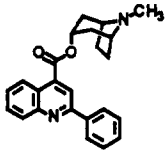
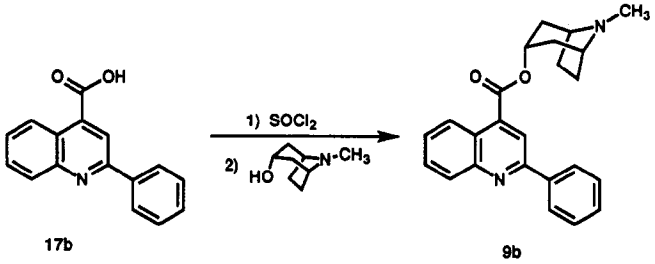


Table I. 2-Substituted Derivatives

compd	structure	yield, %	mp, °C	formula	anal.[a]
9a		20	185.5–188.0	$C_{22}H_{28}N_2O_2 \cdot C_4H_4O_4^b \cdot 2.0H_2O$	C, H, N
9b		30	148.0–149.0	$C_{24}H_{24}N_2O_2 \cdot 2HCl \cdot 2.0H_2O$	C, H, N

[a] Analyses for the elements indicated were within ±0.4% of the theoretical values. [b] $C_4H_4O_4$, fumaric acid.

Scheme II



5-HT- and TRH-Induced Defecation, Castor Oil-Induced Diarrhea and Wrap-Restraint Stress-Induced Colonic Propulsion in Rats. With respect to the selected compounds (**1**, **2**, **4**, **6a**, and **10e**), effects on 5-HT- and TRH-induced defecation[16,28] and castor oil-induced diarrhea[29] were examined (Table IV). An increase in pellet weight was induced in a dose-dependent manner by subcutaneous administration of 5-HT at doses ranging from 1 to 10 mg/kg. Compounds **10e** and **4** significantly inhibited 5-HT (3 mg/kg)-induced increase in fecal pellet output with the ID_{50} values of 0.018 and 0.013 mg/kg, po, respectively. Compounds **6a**, **1**, and **2** also inhibited 5-HT-induced defecation with relatively high doses (ID_{50} = 0.49–0.69 mg/kg, po).

Subcutaneous administration of TRH at 1 mg/kg resulted in an increase in defecation with a 3-h pellet output weight (2–3-fold more stool weight than that in the normal). All compounds showed significant prevention of TRH-induced increases in the excreted stool weight of fed rats. Compound **4** exhibited the most potent activity.

Oral administration of castor oil (1 ml/rat) caused diarrhea. All compounds inhibited castor oil-induced diarrhea with higher doses (IE_{50} = 0.79–1.4 mg/kg) than those in other models.

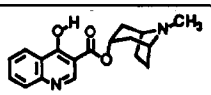
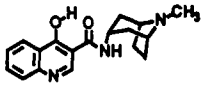
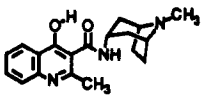
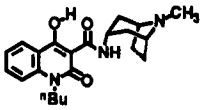
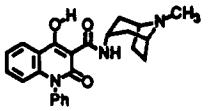
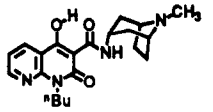
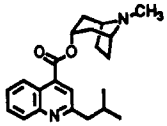
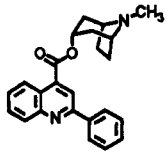
Further, the ability to inhibit wrap-restraint stress-induced colonic propulsion was examined by measuring the time required to expel a Teflon ball which was inserted about 3 cm into the rat colon.[30] Compounds **1** and **6a** did not effectively inhibit this colonic propulsion. However, compounds **10e** and **4** potentially inhibited the propulsion with the ID_{50} values of 0.010 and 0.069 mg/kg, po, respectively.

Discussion

Stress is a complex reaction characterized by the activation of both hormonal and neuronal systems. In humans, stress commonly results in gastrointestinal disorders such as IBS, in association with changes in gastrointestinal motility, gastric secretion, and digestive transit.[17,31,32] Wrap-restraint stress resulted in altered small and large intestinal transit but did not result in diarrhea.[17] No erosions developed after 60 min of wrap-restraint stress. In contrast, cold restraint, which is a method classically used in the study of stress-induced ulcer formation, resulted in gastric erosions. Thus wrap-restraint stress is a mild, nonulcerogenic stressor that reproduces the symptoms associated with stress-related intestinal dysfunction in humans.

Most 1-substituted 2-oxo-1,2-dihydroquinoline-4-carboxylates inhibited wrap-restraint stress-induced defecation. The inhibitory activity of compound **10e** (R = *i*-Bu) was 10–20-fold more potent than that of **1** or **2** and comparable to that of **4** in this model. The present studies appear to confirm the important role of 5-HT and the 5-HT_3 receptor in the wrap-restraint stress. Although compound **6a** has been proved to show the most potent activity in the B-J reflex test (rats, iv) and the cisplatin-induced emesis (dogs, id),[20] **6a** or its derivatives exhibited weak activities in the inhibition of defecation. These results greatly contrast with those of **1**, **2**, **4**, and **10** (Table II). We have previously reported that activity in the B-J reflex test of compounds **6**, **10**, **11**, **12**, and **13** did not have good correlation with the K_i values in the 5-HT_3 receptor binding assay.[19,20] The result in Table III indicates that inhibitory activity of these compounds in a wrap-restraint stress–induced defecation model did not have good correlation with activity in the B-J reflex test. Since this discrepancy might partly be explained by the difference in their pharmacokinetics, selected compounds were further studied in several defecation and diarrhea models.

Table II. 5-HT$_3$ Receptor Binding Affinity, Inhibition of B-J Reflex, and Inhibition of Wrap-Restraint Stress-Induced Defecation for Compounds **1**, **2**, **4**, **5**, **6a,b**, **7a,b**, **8**, and **9a,b**

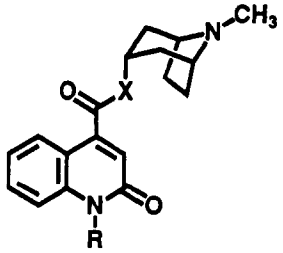
			B-J reflex		defecation	
compd	structure	binding affinity[a] K_i,[b] nM	ED_{50}[c] (mg/kg, iv) at 5 min	95% confidence interval	ID_{50}[d] (mg/kg, po)	95% confidence interval
5		6.1 ● 0.2	0.0017	0.0007–0.0042	(13)	
6a		1.5 ± 0.22	0.00010	0.0000–0.0002	1.4	1.3–1.6
6b		7.2	0.015	0.0098–0.024	(41)	
7a		0.48 ± 0.041	0.29	0.18–0.46	(61)	
7b		1.4 ± 0.23	0.073	0.050–0.14	(48)	
8		6.4	0.29	0.16–0.52	(35)	
9a		0.69 ± 0.06	0.0056		(28)	
9b		(71)[e]	0.34	0.26–0.46	(30)	
1 (ondansetron)		7.6 ± 0.59	0.021	0.015–0.028	0.27	0.23–0.31
2 (granisetron)		2.1 ± 0.26	0.019	0.0056–0.098	0.12	0.10–0.13
4 (YM060)		0.071	0.000089	0.000015–0.00030	0.0052	0.0041–0.0063

[a] [^{3}H]Quipazine-labeled 5-HT$_3$ receptor sites in HG108-15. [b] K_i value was determined as described in the previous paper.[1] Results are expressed as mean values ± SEM after individual measurement (number of determinations is 3). [c] ED_{50}, i.e. the dose of antagonists causing 50% reduction of serotonin effect, was calculated by linear-regression analysis (number of rats is 4). [d] ID_{50}, i.e. the dose of antagonists causing 50% inhibition of restraint stress-induced increase in defecation. Number in a parentheses is inhibition percent at 1 mg/kg, po. [e] Numbers in parentheses are inhibition percents at 10 nM.

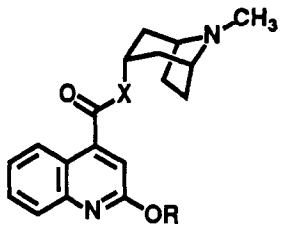
5-HT has been reported to cause an increase in the number of fecal pellets excreted from rats via stimulation of the 5-HT$_3$ receptors. TRH activates colonic transit via a vagally mediated serotonergic mechanism.[28] Thus, compounds **1**, **2**, **4**, **6a**, and **10e** clearly inhibited defecation induced by 5-HT and TRH (Table IV). On the other hand, castor oil-induced diarrhea has been suggested not to be mediated by 5-HT and/or 5-HT$_3$ receptors.[16] Consequently, all 5-HT$_3$ receptor antagonists did not effectively inhibit castor oil-induced diarrhea.

Compound **6a** showed moderate activity in three models (5-HT, TRH, and diarrhea), and its activity was as potent as that of **1** or **2**, but much less potent than that of **4**. On the contrary, compound **10e** exhibited activity as potent as that of **4** in 5-HT- and TRH-induced defecation assays, and 7-fold more potent activity than that of **4** in inhibition of colonic propulsion, although **10e** showed nearly an 800-fold decreased potency compared with **4** in the B-J reflex test. This difference could not be explained by simple difference in their oral absorption because oral administration of **10e** potently inhibited the B-J reflex, and its intravenous administration inhibited colonic repulsion as potently as oral administration. Detailed studies will be published elsewhere. The pharmacological specificity of compounds **4**, **6a**,[20] and **10e** for 5-HT$_3$ recognition sites was examined by investigating them in a wide variety of binding assays. These include assays for serotonin receptors (5-HT$_{1A}$, [^{3}H]-8-OH-DPAT, rat hippocampus), dopamine receptors (D_1, [^{3}H]SCH23390, rat striatum; D_2, [^{3}H]spiperone, rat striatum), adenosine receptors (A_1, [^{3}H]-CHA, guinea pig cerebral cortex; A_2, [^{3}H]NECA, rat striatum), histamine receptors, (H_1, [^{3}H]pyrilamine, guinea pig cerebellum), and adrenaline receptors (α_1, [^{3}H]-WB4104, [^{3}H]clonidine, rat cerebral cortex; β, [^{3}H]-dihydroalprenolol, rat cerebral cortex). The apparent affinities, measured as IC_{50} (concentration of 50% inhibition) values, for **4**, **6a**, and **10e** were larger than 10 μM, demonstrating that these compounds were inactive at displacing binding to a number of recognition sites. Further, **4**, **6a**, and **10e** did not exhibit 5-HT$_4$ agonistic and antagonistic activity at 10 μM in the rat oesophageal

Table III. 5-HT_3 Receptor Binding Affinity, Inhibition of B-J Reflex, and Inhibition of Wrap-Restraint Stress-Induced Defecation for Compounds **10a-10k**, **11a-11c**, **12a-f**, and **13a-c**



10 (X = O)
11 (X = NH)



12 (X = O)
13 (X = NH)

compd	R	binding affinity[a] K_i,[b] nM	B-J reflex ED_{50}[c] (mg/kg, iv) at 5 min	B-J reflex 95% confidence interval	defecation ID_{50}[d] (mg/kg, po)	defecation 95% confidence interval
10a	CH_3	2.6 ± 0.20	0.18	0.085–0.51	(47)	
10b	CH_3CH_2	1.1 ± 0.07	0.022	0.018–0.026	0.052	0.046–0.057
10c	$(CH_3)_2CH$	0.32 ± 0.045	0.081	0.044–0.15	0.040	0.033–0.048
10d	$CH_3(CH_2)_2$	0.45 ± 0.009	0.33	0.12–0.92	0.17	0.16–0.19
10e	$(CH_3)_2CHCH_2$	0.47 ± 0.021	0.033	0.022–0.047	0.013	0.011–0.014
10f	$CH_3(CH_2)_3$	0.68 ± 0.036	0.041	0.015–0.11	0.14	0.12–0.16
10g	$(CH_3)_2CH(CH_2)_2$	1.5 ± 0.0	0.059	0.044–0.078	0.041	0.035–0.047
10h	$CH_3(CH_2)_4$	0.96 ± 0.09	0.059	0.044–0.081	0.073	0.066–0.080
10i	$CH_3(CH_2)_5$	1.1 ± 0.07	0.086	0.067–0.11	0.066	0.057–0.076
10j	$C_6H_5CH_2$	1.3 ± 0.05	0.15	0.12–0.20	(22)	
10k	C_6H_5	0.51 ± 0.012	0.044	0.035–0.057	(59)	
11a	$CH_3(CH_2)_2$	67 ± 16	0.35	0.27–0.46	(12)	
11b	$CH_3(CH_2)_3$	60 ± 4.9	0.37	0.26–0.55	(47)	
11c	C_6H_5	63 ± 9	0.19	0.14–0.26	(14)	
12a	CH_3	0.58 ± 0.015	0.42	0.21–0.82	(45)	
12b	CH_3CH_2	0.88 ± 0.025	0.070	0.049–0.12	(36)	
12c	$(CH_3)_2CHCH_2$	0.44 ± 0.064	0.24	0.18–0.31	(36)	
12d	$CH_3(CH_2)_3$	0.39 ± 0.020	0.20	0.11–0.31	(64)	
12e	$CH_3(CH_2)_4$	0.86 ± 0.10	1.13	0.93–1.39	(30)	
12f	$CH_3(CH_2)_5$	3.2 ± 0.26	7.68	3.48–16.9	(23)	
13a	$CH_3(CH_2)_2$	42 ± 11	1.54	0.90–2.63	(29)	
13b	$(CH_3)_2CH$	53 ± 19	1.86	1.13–3.04	(39)	
13c	$CH_3(CH_2)_3$	19 ± 25	0.54	0.43–0.74	(43)	
1 (ondansetron)		7.6 ± 0.59	0.021	0.015–0.028	0.27	0.23–0.31
2 (granisetron)		2.1 ± 0.26	0.019	0.0056–0.098	0.12	0.10–0.13
4 (YM060)		0.071 ± 0.0071	0.000089	0.000015–0.00030	0.0052	0.0041–0.0063

[a-d] See footnotes *a-d* in Table II.

Table IV. Effects of **6a**, **10e**, and Reference Compounds on 5-HT- and TRH-Induced Defecation, Castor Oil-Induced Diarrhea, and Wrap-Restraint Stress-Induced Colonic Propulsion

compd	ID_{50}[a] [mg/kg, po] inhibition of defecation 5-HT	ID_{50}[a] [mg/kg, po] inhibition of defecation TRH	ID_{50}[a] [mg/kg, po] inhibition of diarrhea castor oil	ID_{50}[b] [mg/kg, po] inhibition of colonic propulsion
6a (KF 20170)	0.69 (0.63–0.76)	0.053 (0.044–0.063)	0.79 (0.52–1.3)	1.8 (1.7–1.9)
10e (KF 18259)	0.018 (0.016–0.021)	0.021 (0.019–0.025)	1.4 (0.95–2.3)	0.010 (0.0092–0.011)
1 (ondansetron)	0.53 (0.48–0.59)	0.31 (0.26–0.36)	1.4 (1.2–1.7)	1.6 (1.5–1.7)
2 (granisetron)	0.49 (0.44–0.56)	0.074 (0.066–0.082)	0.91 (0.74–1.1)	0.74 (0.67–0.82)
4 (YM060)	0.013 (0.011–0.015)	0.0051 (0.0046–0.0057)	1.1 (0.77–1.9)	0.069 (0.065–0.074)

[a] ID_{50}, i.e. the dose of antagonists causing 50% inhibition of increase in defecation and castor oil-induced diarrhea. Numbers in parentheses are 95% confidence intervals. [b] ID_{50}, i.e. the dose of antagonists causing 50% inhibition of decrease in the time of expulsion of a Teflon ball. Numbers in parentheses are 95% confidence intervals.

tunica muscularis mucosae.[33] Thus, **4**, **6a**, and **10e** were identified to be selective 5-HT_3 antagonists.

The B-J reflex is known to be mediated by reflex stimulation of the vagus nerve following activation of the sensory nerve located in the right ventricle wall.[19] Wrap-restraint stress-induced defecation and colonic propulsion could be mediated by the 5-HT_3 receptor in the gastrointestinal system. Therefore, compound **10e** seems to interact selectively with the 5-HT_3 receptor in the gastrointestinal system and might be different from the reported 5-HT_3 receptor antagonists, including **4**, which are considered to interact with both of the 5-HT_3 receptor in the gastrointestinal system and that in the cardiovascular system. Considering the structure of compound **10e**, its carbonyl moiety seems to deviate from the plane of an aromatic ring in the minimum-energy conformer, similarly as in the case of compound **10a**.[19] The carbonyl moiety of **11a** also deviates from the planarity, but a series of **11** showed decreased affinity for the 5-HT_3 receptor and did not inhibit stress-induced defecation. Thus this deviation might be important for **10** as a 5-HT_3 antagonist in the gastrointestinal system. Compound **10e** may belong to a novel class of 5-HT_3 receptor antagonists. Different properties of 5-HT_3 receptor antagonists in these models

suggested that there are multiple subtypes of the 5-HT$_3$ receptor. In fact, the existence of such multiple 5-HT$_3$ receptor molecular species has been suggested by several groups.[34,35]

In conclusion, wrap-restraint stress as well as exogenous 5-HT or TRH administration caused significant alterations in bowel function in rats. KF 18259 (**10e**), which possesses high affinity for the 5-HT$_3$ receptor but showed moderate activity in the B-J reflex test, inhibited potently wrap-restraint stress-, 5-HT-, TRH-induced defecation and wrap-restraint stress-induced colonic propulsion. Since **10e** might be a selective 5-HT$_3$ antagonist in the gastrointestinal system, this class of 5-HT$_3$ receptor antagonists might be effective in the therapy of IBS.

Experimental Section

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a JEOL JNM GX-270 FT NMR or a Hitachi R-90H FT NMR spectrometer with Me_4Si as an internal standard, and mass spectra were recorded on a JMS-SX102 instrument. Melting points were determined with a Büchi-510 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a JASCO IR-810 spectrometer. Elemental analysis were performed by the analytical department of our laboratories.

Chemistry. *endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 2-Isobutyl-4-quinolinecarboxylate Fumarate (9a). To a mixture of KOH (44.0 g, 784.2 mmol) and EtOH (400 mL) was added isatin (**16**; 22.1 g, 150.2 mmol) and subsequently 4-methyl-2-oxopentane (40 mL, 319.5 mmol) in EtOH (100 mL), followed by heating under reflux for 30 min. After cooling and concentration of the mixture under reduced pressure, Et_2O was added to the residue followed by extraction with H_2O. Then, dilute HCl was added to acidify the solution. The precipitated crystals were collected by filtration, washed with H_2O, and dried to give compound **17a** (13.3 g, 39%): ^{1}H NMR (DMSO-d_6) δ 8.63 (d, 1 H, J = 8.5 Hz), 8.05 (d, 1 H, J = 8.2 Hz), 7.81 (s, 1 H), 7.50–7.85 (m, 2 H), 2.86 (d, 2 H, J = 7.0 Hz), 2.21 (m, 1 H), 0.93 (d, 6 H, J = 6.6 Hz). $SOCl_2$ (20 mL) was added to the compound described above (2.81 g, 12.3 mmol) followed by stirring. Excess $SOCl_2$ was removed under reduced pressure, and anhydrous THF (50 mL) was added (solution A). A 15% n-BuLi-hexane solution (8.8 mL, 14.2 mL) was added to a mixture of tropine (2.00 g, 14.2 mmol) and anhydrous THF (6 mL) at 0 °C followed by stirring for a further 15 min. Then, solution A was dropwise added under an argon atmosphere. The mixture was stired at 0 °C for 1 h and at room temperature for a further 2.5 h. To this mixture was added a small quantity of MeOH and H_2O followed by extraction with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography with $CHCl_3$-MeOH (10:1) as eluent. To this product were added i-PrOH (40 mL) and fumaric acid (0.26 g, 2.24 mmol), and the mixture was stirred at room temperature. Hexane (15 mL) was added to this solution at room temperature with stirring, and the precipitated crystals were collected by filtration and dried to give compound **9a** as the fumarate (1.16 g, 20%): IR (KBr) 3410 (br), 2958, 1715, 1694, 1590, 1367, 1273, 1254, 1201, 1158, 1079, 1024, 981, 970, 928, 823, 798, 774, 651, 633, 550 cm^{-1}; MS m/z 352 (M$^+$); ^{1}H NMR (DMSO-d_6) δ 8.63 (d, 1 H, J = 7.7 Hz), 8.07 (d, 1 H, J = 7.7 Hz), 7.83 (s, 1 H), 7.81 (m, 1 H), 7.71 (m, 1 H), 5.32 (m, 1 H), 3.69 (m, 2 H), 2.90 (d, 2 H, J = 7.3 Hz), 2.59 (s, 3 H), 2.00–2.65 (m, 9 H), 0.96 (d, 6 H, J = 6.6 Hz). Anal. ($C_{22}H_{28}N_2O_2 \cdot C_4H_4O_4$) C, H, N.

***endo*-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 2-Phenyl-4-quinolinecarboxylate Dihydrochloride (9b).** The product in a free form obtained in a similar manner to that described above was dissolved in $CHCl_3$, and to this solution was added EtOAc saturated with HCl. The mixture was poured into cold Et_2O with stirring, and the precipitated crystals were collected by filtration and dried to give compound **9b** as the hydrochloride (30%): MS m/z 372 (M$^+$); ^{1}H NMR (DMSO-d_6) δ 11.13 (br s, 1 H), 8.65 (d, 1 H, J = 7.8 Hz), 8.50 (s, 1 H), 8.24–8.36 (m, 2 H), 8.22 (d, 1 H, J = 7.8 Hz), 7.90 (m, 1 H), 7.75 (m, 1 H), 7.51–7.68 (m, 3 H), 5.38 (m, 1 H), 3.93 (m, 2 H), 2.68 (d, 3 H, J = 4.9 Hz), 2.05–2.90 (m, 8 H). $C_{24}H_{24}N_2O_2 \cdot 2HCl$.

Biology. 5-HT$_3$ Receptor Binding Test. The binding assay was carried out according to the procedure described in the previous papers.[19,20]

Pharmacology. Bezold–Jarisch Reflex Test. The Bezold-Jarisch reflex assay was carried out according to the reported procedure.[36] Male Wistar rats (230–330 g, Japan SLC) were anesthetized with urethane (1.25 g/kg, ip), and their tracheas were cannulated. Blood pressure was recorded from the left carotid artery via a saline/heparin-filled pressure transducer from which the heart rate was also continuously monitored. Compounds dissolved in saline were injected intravenously into the exterior jugular vein. The B-J reflex was evoked by a rapid bolus iv injection of 5-HT (30 μg/kg), and consistent responses were established every 12 min before each challenge with 5-HT. Each compound was evaluated at 3–6 doses (n = 4 in each dose). The ED_{50} values, the doses which reduced the 5-HT-induced bradycardia by 50%, and 95% of confidence intervals were calculated by Probit method and Lichfield–Wilcoxon method.

Effects on Wrap-Restraint Stress-Induced Defecation.[17] Adult male Sprague–Dawley (SD) rats weighing 150–200 g were given the test compounds orally. After 60 min, rats were lightly anesthetized with ether, and their foreshoulders, upper forelimbs, and thoracic trunk were wrapped in paper tape to restrict, but not prevent, movement. Rats recovered from ether within 2–5 min and immediately moved about in their cages and ate and drank. The wrap-restraint restricted mobility of their forelimbs, which prevented them from grooming the face, upper head, and neck. Normal rats were anesthetized with ether but were not wrapped. The effects of the test compounds were determined by measuring the stool weight with 60 min of stressing. The stool weights of control rats (vehicle treated) and normal rats (without stress) were 0.4–0.6 and 0.1–0.2 g/rat, respectively. The test compounds were evaluated at four doses (n = 12 in each dose). The ID_{50} values and 95% of confidence interval were calculated by Probit method.

Effects on 5-HT-Induced Defecation.[16] Adult male SD rats weighing 150–200 g were given the test compounds orally. After 60 min, 5-HT was administered subcutaneously (3 mg/kg). The effects of the test compounds were determined by measuring the stool weight 60 min after 5-HT administration. The stool weights of control (vehicle treated) and normal rats (without 5-HT) were 0.6–0.8 and 0.2–0.25 g/rat, respectively. The test compounds were evaluated at four doses (n = 12 in each dose). The ID_{50} values and 95% of confidence interval were calculated by Probit method.

Effects on TRH-Induced Defecation.[16] Adult male SD rats weighing 150–200 g were given the test compounds orally. After 60 min, TRH was administered subcutaneously (1 mg/kg). The effects of the test compounds were determined by measuring the stool weight 3 h after TRH administration. The stool weights of control rats (vehicle treated) and normal rats (without TRH) were 0.5–0.55 and 0.2–0.3 g/rat, respectively. The test compounds were evaluated at five doses (n = 12 in each dose). The ID_{50} values and 95% of confidence interval were calculated by Probit method.

Effects on Castor Oil-Induced Diarrhea.[29] Adult male SD rats weighing 150–200 g were given the test compounds orally. After 60 min, castor oil was administered orally (1 ml/rat). The effects of the test compounds were determined by measuring the stool weight 90 min after castor oil administration. The stool weights of control rats (vehicle treated) and normal rats (without castor oil) were 1.5–2.0 and 0 g/rat, respectively. The test compounds were evaluated at four doses (n = 8 in each dose). The ID_{50} values and 95% of confidence intervals were calculated by Probit method.

Effects on Wrap-Restraint Stress-Induced Colonic Propulsion.[30] Adult male SD rats weighing 150–200 g were given the test compounds orally. After 60 min, rats were lightly anesthetized with ether, stressed by wrapping their foreshoulders, upper forelimbs and thoracic trunk in paper tape, and then a Teflon ball (diameter = 3.17 mm) was inserted about 3 cm into the rat colon. The effects of the test compounds were determined by measuring the time required to expel a Teflon ball. The times in control rats (vehicle treated) and normal rats (without wrap

restraint) were 150–300 and 1800–2300 seconds, respectively. The test compounds were evaluated at four doses (n = 10 in each dose). The ID_{50} values and 95% of confidence intervals were calculated by Probit method.

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